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Supporting Information

Photoenhanced Oxidative DNA cleavage with Non-Heme Iron(II) Complexes

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Experimental set-up of DNA cleavage

Reactions were carried out in 1.5 mL eppendorfs with open caps thermostatted at 37 ºC in the dark. Light beams covered the whole area of top surface of reaction solutions and passed through the reaction solutions vertically. Figure S1 shows the set-up.

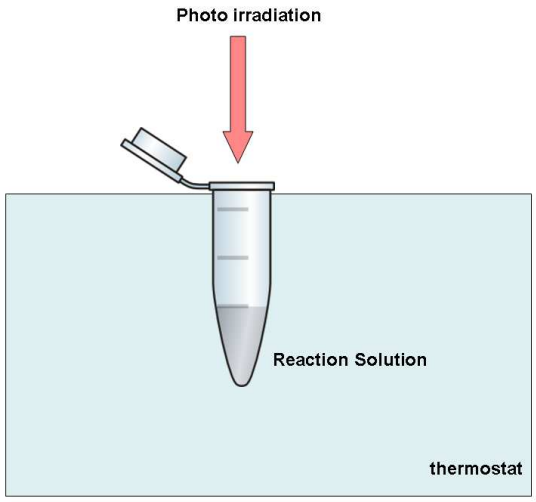

Figure S1: Experimental set-up for DNA cleavage experiments

Determination of irradiation power

The iron(III) oxalate/phenanthroline actinometer system was used to determine the light flux (*R*) of irradiation.[1] One example is shown in Figure S2.

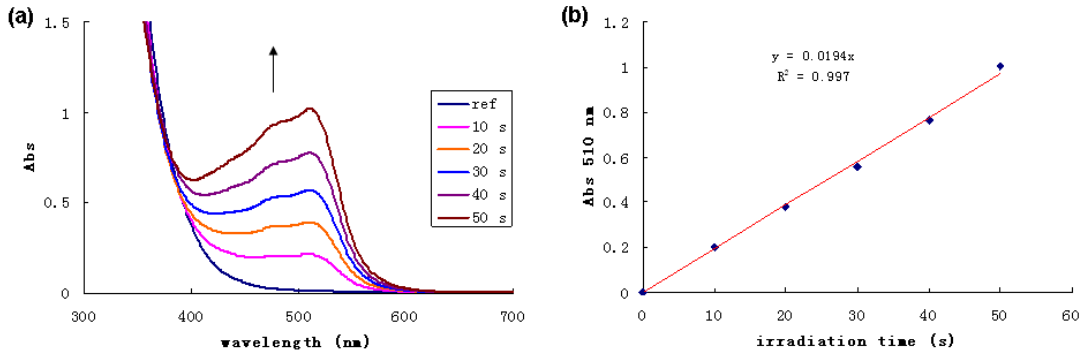

Figure S2: For CW laser 355 nm (3 mW), (a) absorption spectra of iron(III) oxalate (different irradiation time)/phenanthroline aqueous solutions; (b) absorption of iron(III) oxalate (different irradiation time)/phenanthroline aqueous solutions at 510 nm.

The power (*P*) at the sample was calculated using Equation (S1), in which E_p is the energy of one photon, *h* is Planks constant (6.626×10^{-34} Js), *c* is the speed of light (3.0×10^{8} ms^{-1}), λ is the wavelength of light source (355, 400.8 and 473 nm, respectively). Table S1 shows the light flux (*R*) and power (*P*) at sample under different irradiation conditions.

$$P = E_p R = \frac{\hbar c}{\lambda} R \quad \text{(S1)}$$

Table S1: Light flux (*R*) and power (*P*) of different light sources at sample.

	Pulsed Laser		CW laser				
	355 nm		355 nm		400.8 nm		473 nm
R (*photons.s*$^{-1}$)	5.7 × 10^{15}	4.4 × 10^{16}	5.2 × 10^{15}	4.7 × 10^{15}	3.5 × 10^{16}	2.3 × 10^{15}	7.2 × 10^{16}
P (*mW*)	3.2	24.6	2.9	2.6	17.4	1.2	30.3

UV-Vis absorption spectra of Fe(II).L

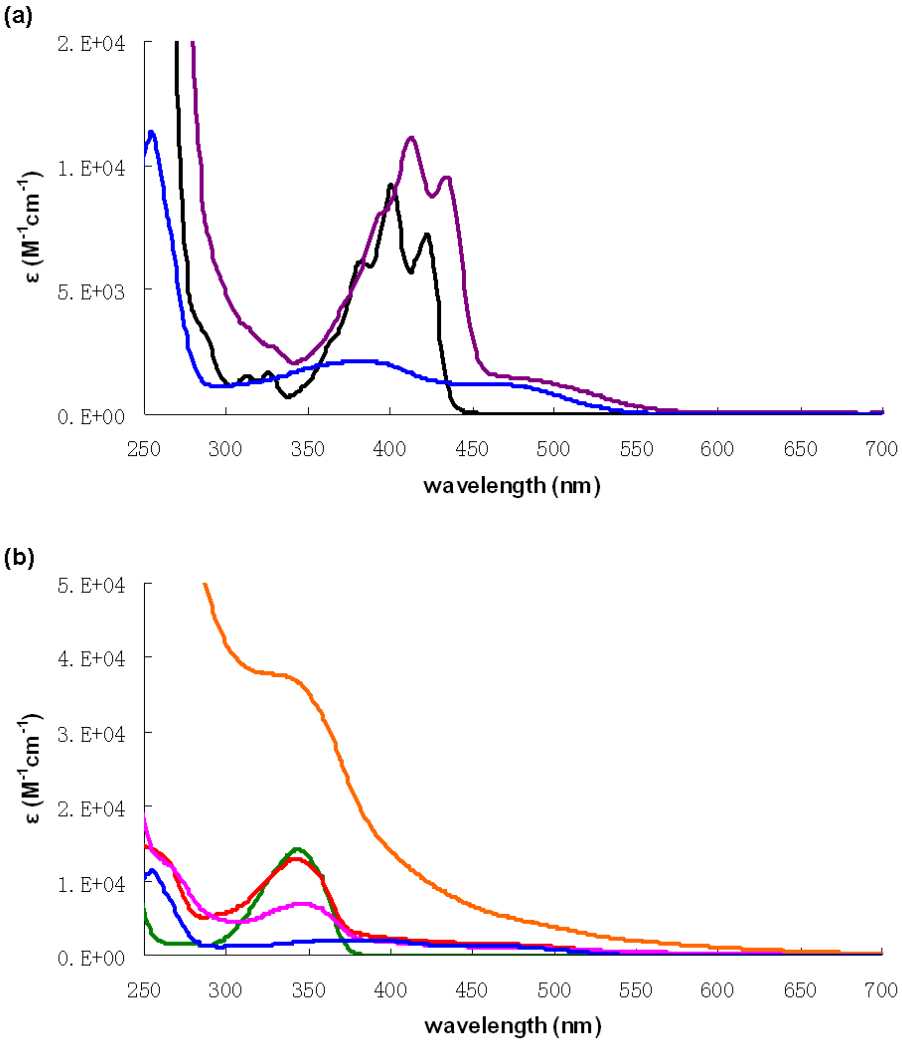

Figure S3: Normalized absorption spectra of (a) 9-aminoacridine (—), Fe(II)-**1** (—) and Fe(II)-**2** (—); (b) Fe(II)-**1** (—), 1,8-naphthalimide (—), Fe(II)-**3** (—),Fe(II)-**4** (—) and Fe(II)-**5** (—).

DNA cleavage under photo irradiation

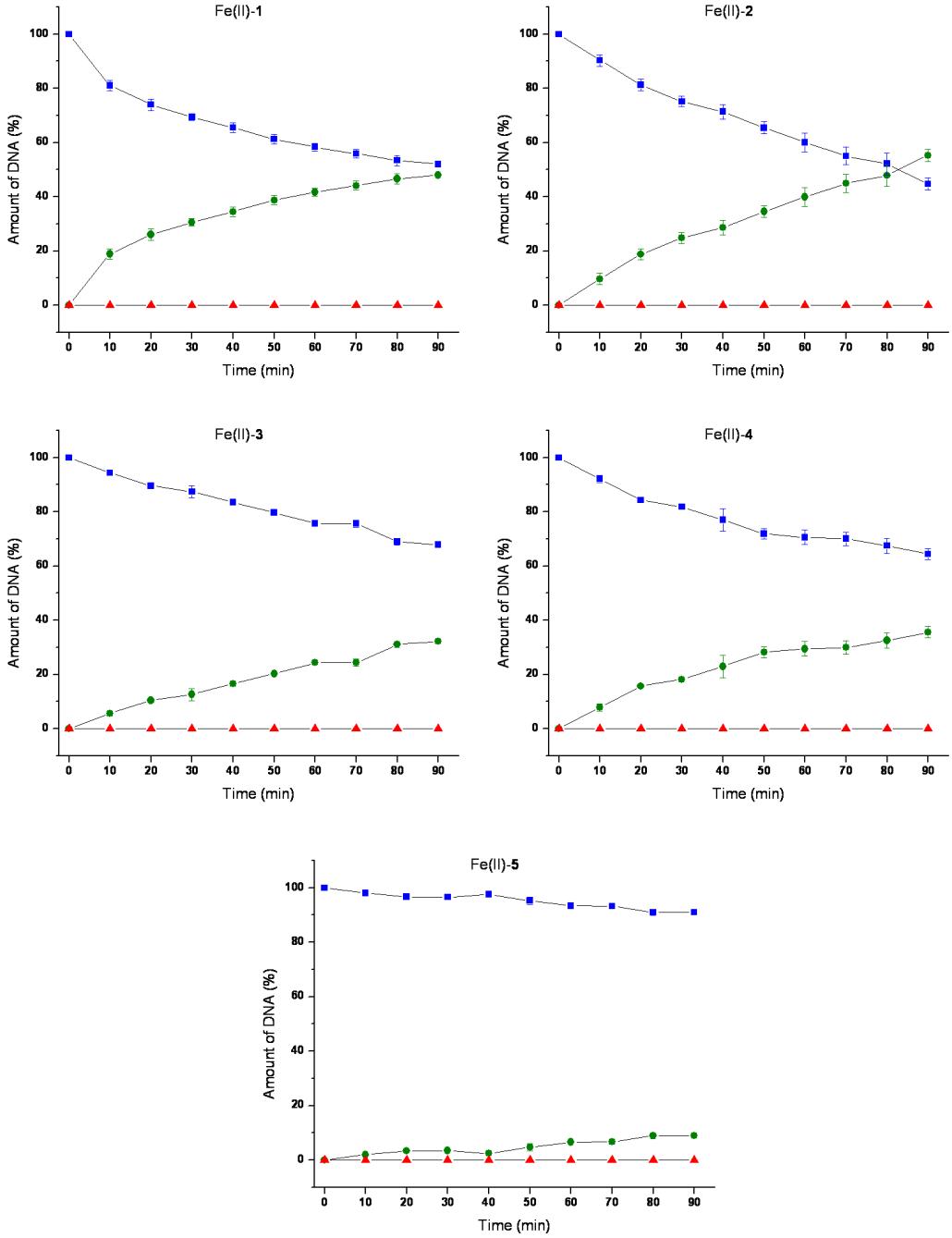

Figure S4: Time profile for cleavage of supercoiled DNA (■) to nicked (●) and linear (▲) DNA in 10 mM Tris-HCl (pH 8.0) at 37 ºC with Fe(II) complexes of ligands **1-5** under photo irradiation at 473 nm (30.3 mW). Conditions: 1.0 µM complex, 0.1 µg/µl pUC18 plasmid DNA (150 µM bp).

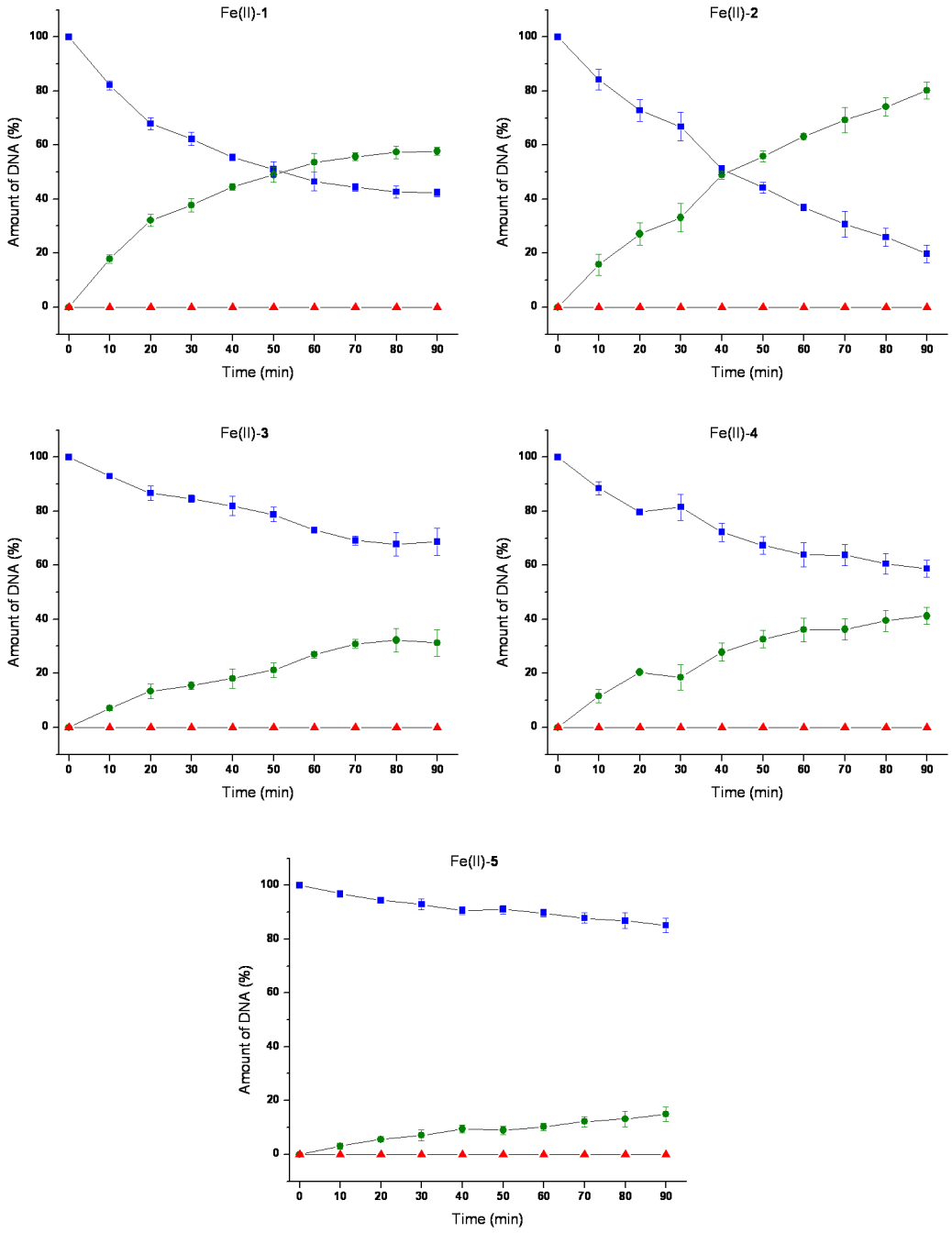

Figure S5: Time profile for cleavage of supercoiled DNA (■) to nicked (●) and linear (▲) DNA in 10 mM Tris-HCl (pH 8.0) at 37 ºC with Fe(II) complexes of ligands **1-5** under photo irradiation at 400.8 nm (17.4 mW). Conditions: 1.0 µM complex, 0.1 µg/µl pUC18 plasmid DNA(150 µM bp).

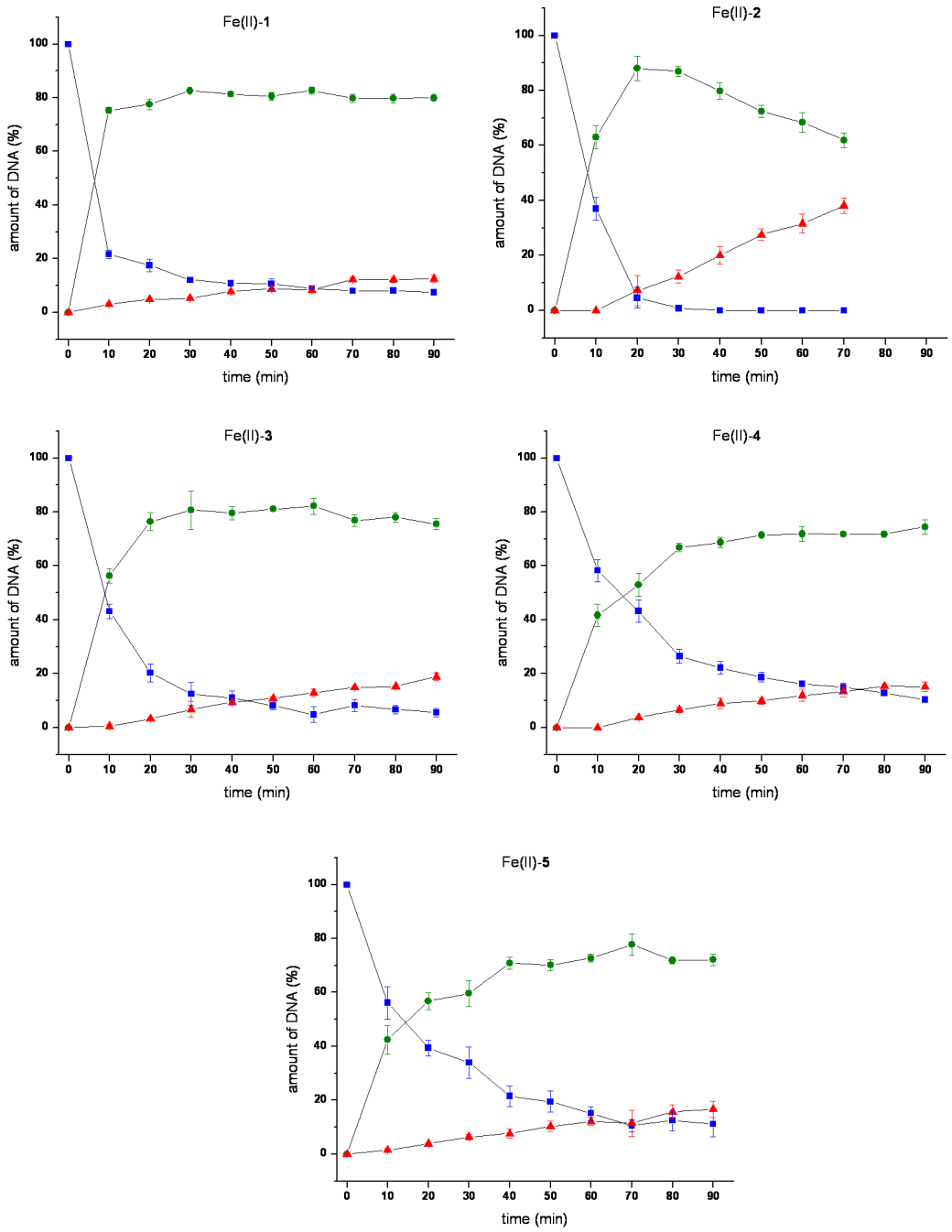

Figure S6: Time profile for cleavage of supercoiled DNA (■) to nicked (●) and linear (▲) DNA in 10 mM Tris-HCl (pH 8.0) at 37 ºC with Fe(II) complexes of ligands **1-5** under photo irradiation at 355 nm (24.6 mW). Conditions: 1.0 µM complex, 0.1 µg/µl pUC18 plasmid DNA (150 µM bp).

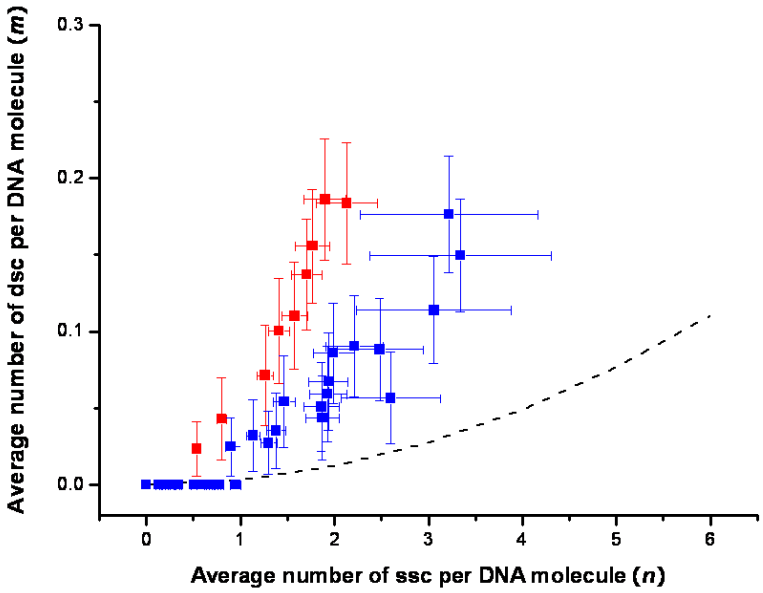

Figure S7: Number of double-strand cuts (*m*) as a function of single-strand cuts (*n*) per DNA molecule for Fe(II)-**4** in the presence of 1000 equiv. DTT (■) and under pulsed irradiation at 355 nm (24.6 mW) (■). Error bars represent the maximum and the minimum values of *n* and *m*. Dotted lines describe a pure single-strand cleavage pathway, as described by the Freifelder-Trumbo relationship.[3]

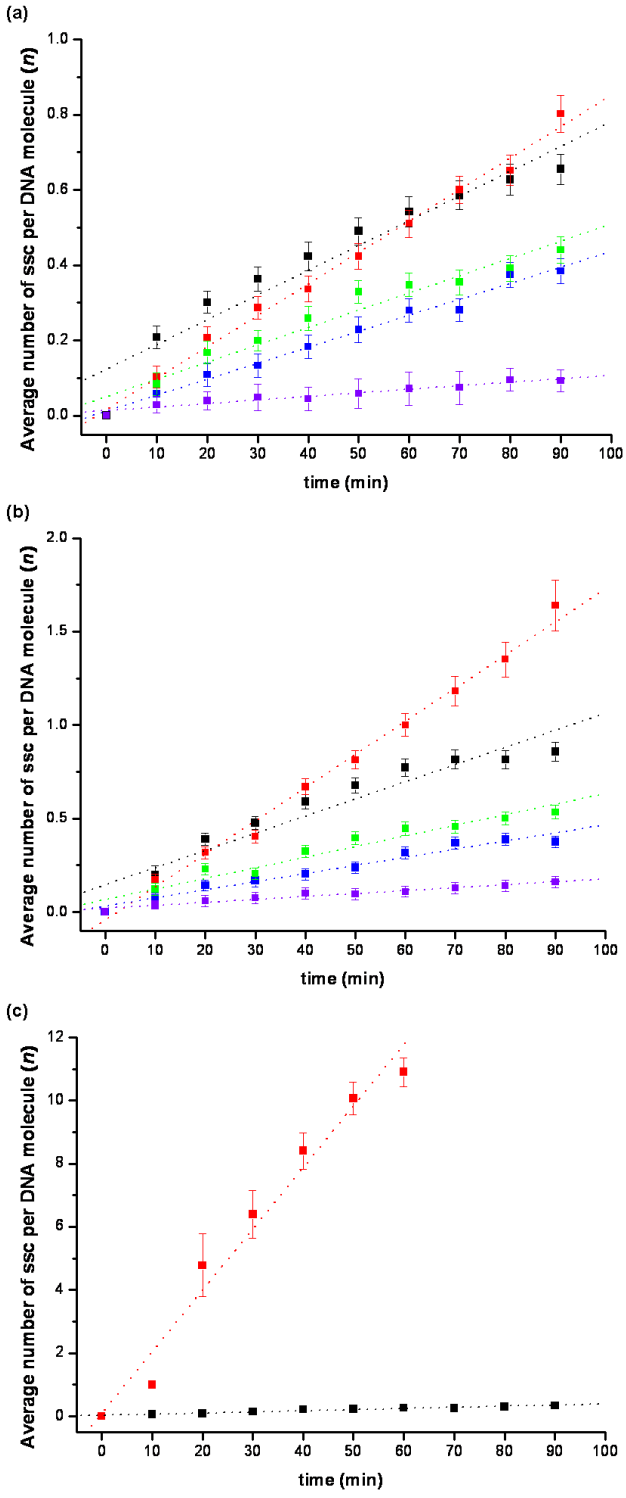

Figure S8: Number of single-strand cuts (*n*) as a function of time per DNA molecule for (a) Fe(II)-**1** (■), Fe(II)-**2** (■), Fe(II)-**3** (■), Fe(II)-**4** (■) and Fe(II)-**5** (■) under photo irradiation at 473 nm (30.3 mW); (b) Fe(II)-**1** (■), Fe(II)-**2** (■), Fe(II)-**3** (■), Fe(II)-**4** (■) and Fe(II)-**5** (■) under photo irradiation at 400 nm (17.4 mW); (c) Fe(II)-**2** under ambient lighting (■) and photo irradiation at 355 nm (24.6 mW) (■). Dashed lines represent the linear fit through the data points. Conditions: 1.0 µM complex, 0.1 µg/µl pUC18 plasmid DNA (150 µM bp), 10 mM Tris-HCl (pH 8.0), 37 ºC. Dashed lines represent the linear fit through the data points.

Table S2: Rate constants of DNA cleavage (k_{obs}) in the absence and presence of photo irradiation.

reagents	Cleavage rate (min^{-1}) Ambient lighting	Cleavage rate (min^{-1}) 473 nm (30.3 mW)	Cleavage rate (min^{-1}) 400.8 nm (17.4 mW)	Cleavage rate (min^{-1}) 355 nm (24.6 mW)
Fe^{II}-**1**	0.0035 ± 0.0003[a] (R^2 = 0.947)	0.0066 ± 0.0006 (R^2 = 0.9296)	0.0092 ± 0.0010 (R^2 = 0.9154)	[c]
Fe^{II}-**2**	0.0035 ± 0.0001 (R^2 = 0.979)	0.0084 ± 0.0002 (R^2 = 0.9934)	0.0177 ± 0.0006 (R^2 = 0.9922)	0.196 ± 0.015[d] (R^2 = 0.9721)
Fe^{II}-**3**	0.0028 ± 0.0002[a] (R^2 = 0.965)	0.0043 ± 0.0002 (R^2 = 0.9871)	0.0044 ± 0.0003 (R^2 = 0.9649)	[c]
Fe^{II}-**4**	[b]	0.0046 ± 0.0004 (R^2 = 0.9539)	0.0057 ± 0.0005 (R^2 = 0.9482)	[c]
Fe^{II}- **5**	[b]	0.0009 ± 0.0001 (R^2 = 0.9397)	0.0016 ± 0.0001 (R^2 = 0.9582)	[c]

1 μM iron complex, 0.1 μg μL^{-1} supercoiled pUC18 DNA (150 μM bp), Tris-HCl buffer (pH 8.0), 37 ºC. A correction factor of 1.31 is used for the reduced EtBr uptake capacity of supercoiled plasmid pUC18 DNA. [a]The cleavage rate was obtained within 60 min;[4] [b]The cleavage rate can not be obtained through the small numbers of single-strand cuts;[4] [c]The cleavage rate can not be obtained since double-strand cleavage pathway was involved; [d]The cleavage rate was obtained within 60 min, which is before the DNA cleavage process reaches the limit of accurate quantification.

Fluorescence emission spectra of 3-5 and Fe(II)-3-5

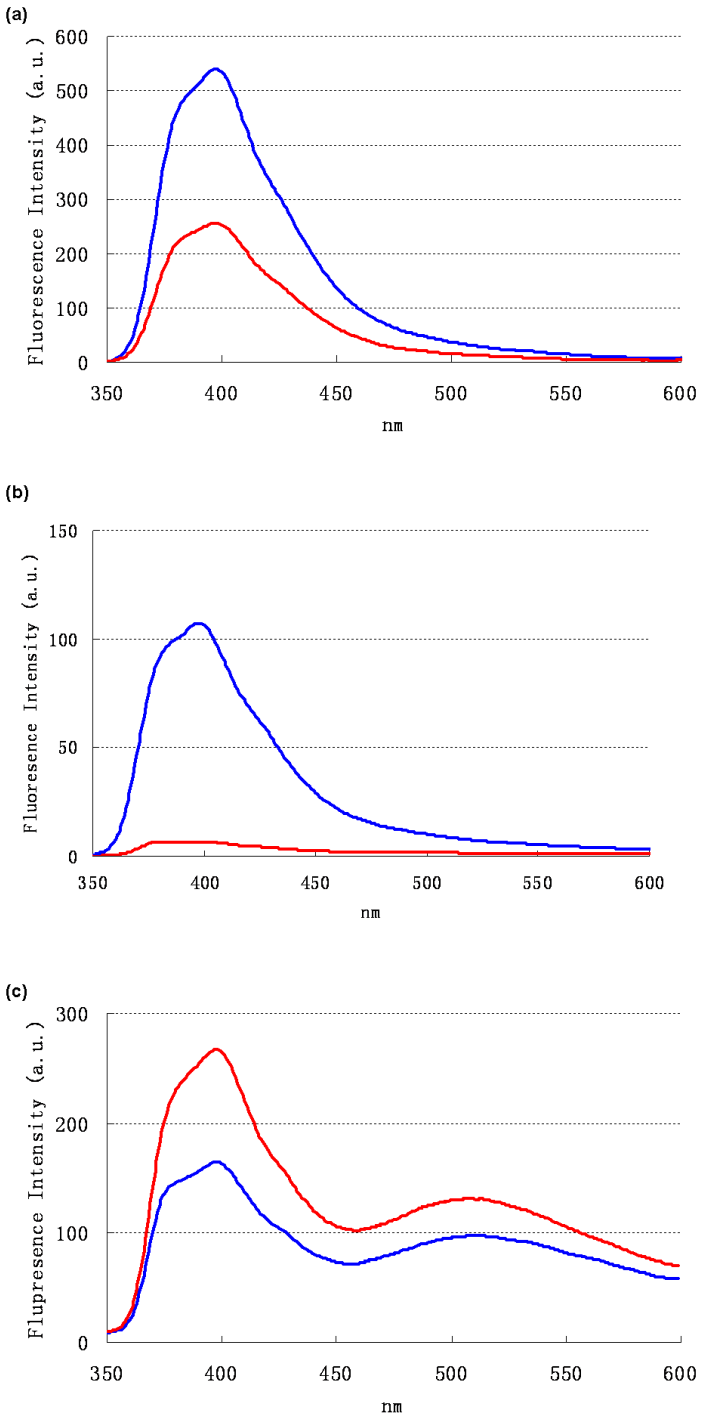

Figure S9: Fluorescence emission spectra in 10 mM Tris-HCl buffer (pH 8.0) at 25 ºC for (a) 1 µM **3** (—) and Fe(II)-**3** (—); (b) 8 µM **4** (—) and Fe(II)-**4** (—); (c) 1 µM **5** (—) and Fe(II)-**5** (—).

Mechanistic investigation on Fe(II)-1

Table S3: Numbers of single-strand cuts per DNA molecule (*n*) with Fe(II)-1 at 30 min with different ROS scavengers.

conditions	*n*				
	No additive	NaN_3	DMSO	SOD	SOD + catalase
Ambient lighting	0.285 ± 0.032	0.271 ± 0.032	0.263 ± 0.035	0.175 ± 0.031	0.080 ± 0.030
355 nm (25 mW)	2.09 ±0.24 *(0.056 ± 0.030)*[*]	1.06 ± 0.07 *(0.029 ± 0.022)*[*]	1.67 ± 0.14 *(0.045 ± 0.028)*[*]	1.05 ± 0.07 *(0.035 ± 0.025)*[*]	1.00 ± 0.06
400.8 nm (17 mW)	0.474 ± 0.034	0.355 ± 0.031	0.365 ± 0.032	0.372 ± 0.033	0.130 ± 0.033
473 nm (30 mW)	0.365 ± 0.034	0.306 ± 0.033	0.218 ± 0.031	0.264 ± 0.030	0.118 ± 0.029

[*] Number of double-strand DNA cleavage per DNA molecule (*m*).

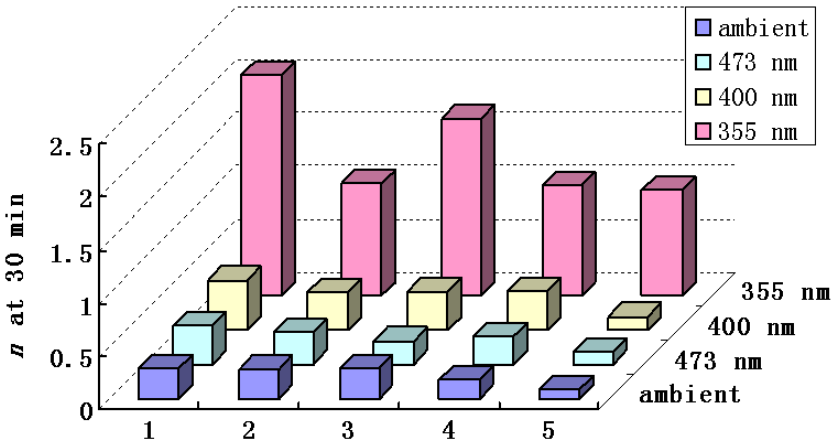

Figure S10: Calculated average numbers of single-strand cuts per DNA molecule (*n*) of Fe-**1** at 30 min with: (1) no ROS scavengers; (2) NaN_3; (3) DMSO; (4) SOD; (5) SOD + catalase. Conditions: 1.0 μM complex, 0.1 μg/μl pUC18 plasmid DNA (150 μM bp), 10 mM Tris-HCl (pH 8.0), 37 ºC; ambient lighting, irradiation at 355 nm (pulsed laser, 24.6 mW), 400.8 nm (CW laser, 17.4 mW) and 473 nm (CW laser, 30.3 mW), respectively.

(1) Montalti, M.; Credi, A.; Prodi, L.; Gandolfi, M. T. *Handbook of Photochemistry*, 3rd ed.; CRC Press: Boca Raton, FL, **2006**.

(2) van den Berg, T. A.; Feringa, B. L.; Roelfes, G. *Chem. Commun.* **2007**, 180-182.

(3) Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681-693.

(4) Li, Q.; van den Berg, T. A.; Feringa, B. L.; Roelfes, G. *Dalton Trans.* **2010**, *39*, 8012-8021.